B1

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
9 December 2004 (09.12.2004)

PCT

(10) International Publication Number
WO 2004/105761 A1

(51) International Patent Classification[7]: A61K 31/495, 31/282, A61P 35/00

(21) International Application Number: PCT/GB2004/002319

(22) International Filing Date: 1 June 2004 (01.06.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0312407.0 29 May 2003 (29.05.2003) GB

(71) Applicant *(for all designated States except US)*: **PHARMA MAR, S.A.U.** [ES/ES]; Polígono Industrial La Mina, Avda. de los Reyes, 1, Colmenar Viejo, E-28770 Madrid (ES).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **D'INCALCI, Maurizio** [IT/IT]; Sendo, Via Visconti di Modrone 12, I-20122 Milan (IT). **GIANNI, Luca** [IT/IT]; Istituto Nazionale dei Tumori, Milan (IT). **GIAVAZZI, Rafaella** [IT/IT]; Sendo, Via Visconti di Modrone 12, I-20122 Milan (IT). **GARCIA MARTIN, Margarita** [ES/ES]; Instituto Catalan de Oncologia, Ctra. Gran Via s/n, E-08907 L'Hospitalet del Llobregat (ES). **JUDSON, Ian** [GB/GB]; Royal Marsden Hospital, 203 Fulham Road, London SW3 6JJ (GB). **JIMENO, Doñaque, Jose, Maria** [ES/ES]; Poligono Industrial La Mina, Avda. de los Reyes, 1, Colmenar Viejo, E-28770 Madrid (ES). **SESSA, Cristiana** [IT/CH]; Ospedale S Giovanni, Bellinzona (CH).

(74) Agent: **RUFFLES, Graham, Keith**; Marks & Clerk, 66-68 Hills Road, Cambridge CB2 1LA (GB).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2004/105761 A1

(54) Title: COMBINED USE OF ECTEINASCIDIN-743 AND PLATINUM ANTINEOPLASTIC COMPOUNDS

(57) Abstract: ET-743 can be used to mitigate resistance to and potentiate the cytotoxic effects of a platinum coordination complex anti-neoplastic agent in a human cancer patient.

COMBINED USE OF ECTEINASCIDIN-743 AND PLATINUM ANTINEOPLASTIC COMPOUNDS

The invention relates to a treatment, more particularly an improved use of antitumoral compounds in cancer therapy.

FIELD OF THE INVENTION

The present invention is directed to the use of ecteinascidin 743 and products containing this compound for cancer therapy, in particular to the use of ecteinascidin 743 in combination with an antineoplastic platinum coordination complex in the treatment of cancer.

BACKGROUND OF THE INVENTION

Cancer comprises a group of malignant neoplasms that can be divided into two categories: carcinoma, comprising a majority of the cases observed in the clinics, and other less frequent cancers, which include leukemia, lymphoma, central nervous system tumours and sarcoma. Carcinomas have their origin in epithelial tissues while sarcomas develop from connective tissues and those structures that had their origin in mesoderm tissues. Sarcomas can affect, for instance, muscle or bone and occur in the bones, bladder, kidneys, liver, lung, parotid, spleen, etc.

Cancer is invasive and tends to metastasise to new sites. It spreads directly into surrounding tissues and also may be disseminated through the lymphatic and circulatory systems.

Many treatments are available for cancer, including surgery and radiation for localised disease, and drugs. However, the efficacy of available treatments on many cancer types is limited, and new, improved forms of treatment showing clinical benefit are needed. This is especially true for those patients presenting with advanced and/or metastatic disease. It is also true for patients relapsing with progressive disease after having been previously treated with established therapies for which further treatment with the same therapy is mostly ineffective due to acquisition of resistance or to limitations in administration of the therapies due to associated toxicities.

Chemotherapy plays a significant part in cancer treatment, as it is required for treatment of advanced cancers with distant metastasis and it is often helpful for tumor reduction before surgery. Many anti-cancer drugs have been developed based on various modes of action.

The most commonly used types of anticancer agents include: DNA-alkylating agents (e. g., cyclophosphamide, ifosfamide), antimetabolites (e. g., methotrexate, a folate antagonist, and 5-fluorouracil, a pyrimidine antagonist), microtubule disrupters (e. g., vincristine, vinblastine, paclitaxel), DNA intercalators (e. g., doxorubicin, daunomycin, cisplatin), and hormone therapy (e. g., tamoxifen, flutamide). The ideal antineoplastic drug would kill cancer cells selectively, with a wide therapeutic index relative to its toxicity towards non-malignant cells. It would also retain its efficacy against malignant cells, even after prolonged exposure to the drug. Unfortunately, none of the current chemotherapies possess an ideal profile. Most possess very narrow therapeutic indexes and, in practically every instance, cancerous cells exposed to slightly sublethal concentrations of a chemotherapeutic agent will develop resistance to

such an agent, and quite often cross-resistance to several other antineoplastic agents.

Combination therapy using drugs with different mechanisms of action is an accepted method of treatment which helps preventing development of resistance by the treated tumor.

The ecteinascidins (herein abbreviated ET or ET's) are exceedingly potent antitumor agents isolated from the marine tunicate Ecteinascidia turbinata. Several ecteinascidins have been reported previously in the patent and scientific literature. See, for example U.S. Pat. No. 5,089,273, which describes novel compounds of matter extracted from the tropical marine invertebrate, *Ecteinascidia turbinata*, and designated therein as ecteinascidins 729, 743, 745, 759A, 759B and 770. These compounds are useful as antibacterial and/or antitumor agents in mammals. U.S. Pat. No. 5,478,932 describes ecteinascidins isolated from the Caribbean tunicate *Ecteinascidia turbinata*, which provide in vivo protection against P388 lymphoma, B16 melanoma, M5076 ovarian sarcoma, Lewis lung carcinoma, and the LX-1 human lung and MX-1 human mammary carcinoma xenografts.

One of them, ecteinascidin-743 (ET-743), is a novel tetrahydroisoquinoline alkaloid isolated from the marine ascidian *Ecteinascidia turbinata* that has considerable *in vitro* and *in vivo* antitumor activity in murine and human tumors, and is presently in clinical trials.

Potent antitumor activity has been demonstrated in a broad range of *in vivo* tumor models, including human tumor xenografts in nude mice. Et-743 has a novel complex mechanism of action at the level of gene transcription. ET-743 binds to guanine-cytosine rich sequences

in the minor groove of DNA and alkylates guanine residues at the N2 position

An *in vitro* bone marrow assay using human, murine and canine progenitor cells, showed equal sensitivity of erythropoid and myeloid cells to ET-743. Prolonged or repeated exposure to the drug proved more toxic to hematopoietic progenitors than a single 1-hour exposure. The therapeutic index of ET-743 was more favourable with prolonged exposure.

A clinical development program of ET-743 in cancer patients was started with phase I studies investigating 1-hour, 3-hour, 24-hour and 72-hour intravenous infusion schedules and a 1 hour daily x 5 (dx5) schedule. In Phase I and II clinical trials ET-743 has shown significant antitumor activity against several human malignancies including soft tissue sarcomas and ovarian carcinomas. Further detail on the use of ET-743 for the treatment of the human body for cancer is given in WO 0069441, incorporated herein by specific reference.

A well known and used family of anticancer agents are the platinum compounds. Cisplatin (cis-diaminedichloroplatinum (II)) is a platinum coordination complex first identified in 1965 as a cytotoxic agent. It has broad activity as an antineoplastic agent and is especially useful in the treatment of epithelial malignancies. Other platinum coordination complexes that have been evaluated in clinical trials include carboplatin, tetraplatin, ormiplatin, iproplatin and oxaliplatin.

The treatment of cancer patients with platinum coordination complex antineoplastic agents, such as cisplatin or carboplatin has increased substantially in the last decade. Cisplatin has proved to be useful in the treatment of multiple malignancies including testicular

cancer, ovarian cancer, and small cell lung cancer, whereas carboplatin has proved to be useful in brain tumors, endometrial cancer, germ cell tumors and head and neck cancer. The mechanism of action is currently unknown but may be related to the ability of these compounds to bind to DNA and form various types of inter- and intrastrand crosslinks that possibly interfere with both DNA and RNA synthesis.

Cancer patients eventually become resistant to treatment with platinum coordination complexes, such as cisplatin or carboplatin. The mechanism of resistance to these compounds is unclear but may be related to decreased drug accumulation, elevation of intracellular concentrations of metallothioneines or glutathione which bind and inactivate the drug, or to decreased drug-DNA adduct formation or repair. Therefore there is a need to develop effective therapies that overcome this resistance.

In cancer cell lines growing in vitro the combination of ET-743 and cisplatin showed an additive or synergistic effect evaluated by isobologram analysis. This synergistic effect has also been confirmed *in vivo*: Erba, E. et al. "ET-743 and cisplatin (DDP) show in vitro and in vivo synergy against human sarcoma and ovarian carcinoma cell lines", Proceed. AACR-NCI-EORTC Nov. 2001, abstract 406; Faircloth, Glynn Thomas, Jr., et al. "In vivo combinations of chemotherapeutic agents with Ecteinascidin 743 (Et743) against solid tumors", Proceed. AACR-NCI-EORTC Nov. 2001, abstract 387; D'Incalci M. et al.: "The combination of ET-743 and cisplatin (DDP): From a molecular pharmacology study to a phase I clinical trial.", proceed. AACR March 2002, abstract 404; D'Incalci, M. et al. "In Human tumor xenografts the resistance to ET-743 or to cisplatin can be overcome by giving the two

drugs in combination.", Proceed. AACR-NCI-EORTC, Nov. 2002, abstract 97.

The combination therapy comprising ET-743 is also the object of WO 02 36135, incorporated herein by specific reference in its entirety.

It is an object of the invention to provide efficacious methods and products for preventing resistance or overcoming established resistance to platinum coordination complex anti-neoplastic agents in human patients. It is another object of the invention to provide an effective method and products for potentiating the cytotoxic effects of platinum coordination complex anti-neoplastic agents in the clinical setting.

SUMMARY OF THE INVENTION

Unexpectedly, we found that when given in combination, the maximum dosage of ET-743 and a platinum compound, particularly cisplatin or carboplatin, can be given without an increase or addition of the toxicity. This has been confirmed in the clinical trials, in which full dose of cisplatin and carboplatin has been given successfully with escalating doses of Et-743.

Thus, the subject invention concerns a novel treatment regimen for cancer patients whereby the platinum compound is administered in combination with ET-743.

The invention further provides a method for treatment of a human cancer patient which involves administering a platinum compound and ET-743, in which the amount of the platinum compound is at least 50%, at least 75%, at least 85%, at least 90%, at least 95 %, or at least

100% of the Recommended Dose for the platinum compound in the absence of ET-743, and the amount of the ET-743 is at least 50%, at least 75%, at least 85%, at least 90%, at least 95 %, or at least 100% of the Recommended Dose for ET-743 in the absence of the platinum compound. The Recommended Doses are based on studies of Dose Limiting Toxicity. Preferably the amounts of the platinum compound and ET-743 are both at least at least 85%, at least 90%, at least 95 %, or at least 100% of the respective Recommended Dose.

In another aspect the present invention is directed to the use of ET-743 in the preparation of a medicament for an effective treatment of a human cancer patient by combination therapy employing ET-743 with a platinum compound, characterised in that the combination overcomes resistance to platinum anti-neoplastic compounds without increasing the toxicity of each drug.

In a related aspect, the invention provides a method of treating a human cancer patient with a platinum compound, wherein ET-743 is administered as a combination therapy without a compensating drop in the dose of the platinum compound.

In yet another embodiment of the present invention, there is provided a method of reducing resistance to platinum anti-neoplastic compounds in an individual having a neoplastic disease comprising administering to an individual ET-743 and the platinum compound in a dosage range which is the same as the dosage given if each of ET-743 and the platinum compound were administered alone.

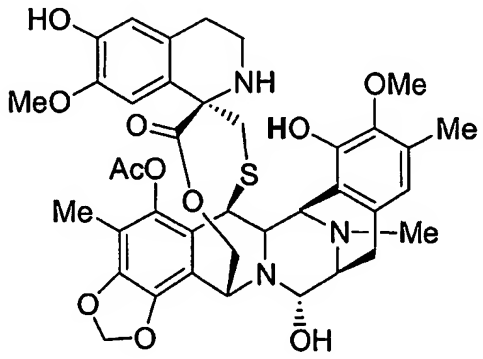
The present invention also provides a pharmaceutical composition containing a recommended dose of ET-743 for weekly administration in

combination with a platinum compound and a pharmaceutically acceptable carrier.

In a further aspect of the present invention, a medical kit for administering ET-743 in combination with an antineoplastic platinum compound is provided, comprising printed instructions for administering ET-743 according to the dosing schedules set forth below, and a supply of ET-743 in dosage units for at least one cycle, wherein each dosage unit contains the appropriate amount of ET-743 for the treatments as defined above and a pharmaceutically acceptable carrier.

DETAILED DESCRIPTION

ET-743 is a natural compound represented by the following formula:



The term "ET-743" is also intended here to cover any pharmaceutically acceptable salt, ester, solvate, hydrate or any other compound which, upon administration to the recipient is capable of providing (directly or indirectly) the compound as described herein. However, it will be appreciated that non-pharmaceutically acceptable salts also fall within the scope of the invention since these may be useful in the preparation of pharmaceutically acceptable salts. The

preparation of salts and prodrugs and derivatives can be carried out by methods known in the art.

ET-743 is supplied and stored as a sterile lyophilized product, consisting of ET 743 and excipient in a formulation adequate for therapeutic use.

The combinations of the present invention comprise ET-743 and an antineoplastic platinum compound, preferably a coordination complex. Preferred complexes include cisplatin, carboplatin, tetraplatin, ormiplatin, iproplatin, oxaliplatin and the like. Preferably, the platinum coordination complex is cisplatin or carboplatin, more preferably cisplatin.

The two drugs can be given simultaneously or one after the other in either sequence, preferably in a sequence.

As it has been said, the invention provides a method for treatment a human cancer patient. Preferably the patients are relapsing or refractory to previous chemotherapy. Most preferably the patients are ovarian cancer, head and neck cancer, NSCL carcinoma or melanoma patients. In a particularly preferred embodiment the patients are ovarian cancer patients and the previous therapy comprises treatment with platinum compounds.

Additionally, the present invention provides a method of treating cancer in humans, comprising a step of intravenously infusing a composition comprising ET-743 into a human having cancer at continuous dosage over a period up to 4 hours, followed or preceded by intravenously infusing a composition comprising a platinum

antineoplastic compound into a human having cancer at a continuous dosage wherein the step of infusing is repeated weekly on a cyclic basis.

The infusing step is typically repeated on a cyclic basis. The cyclic basis comprises two phases, the phase of weekly infusing and a phase of not infusing, referred to as a rest phase. In the rest phase the patients are allowed to recover. Usually the cycle is worked out in weeks, and thus the cycle comprises one or more weeks of an infusion phase, and one or more weeks of a rest phase. The rest period can be longer or shorter than the infusion phase. The preferred duration of each cycle is of 2 to 4 weeks; multiple cycles can be given as needed. Cycles of 3 or 4 weeks with 1 or 2 weeks infusion is most preferred.

When ET-743 is administered in combination with cisplatin, the dosage amount of ET-743 is preferably below 700 $\mu g/m^2$/day on a day 1 & 8 every 3 or 4 weeks schedule, preferably from about 400 to about 650 $\mu g/m^2$/day, more preferably from about 500 to about 650 $\mu g/m^2$/day, even more preferably from about 550 to about 650 $\mu g/m^2$/day. In this case the schedule most preferred is the administration of both compounds on a day 1 & 8 every 4 weeks.

On the other hand, when ET-743 is administered in combination with carboplatin, the dosage of ET-743 is preferably below 1200 $\mu g/m^2$/day on a day 1 every 3 weeks schedule, preferably between 650 and 1200 $\mu g/m^2$/day, more preferably between 800 and 1000 $\mu g/m^2$/day, even more preferably between 800 and 900 $\mu g/m^2$/day.

The dosage amount of cisplatin is the full dosage range used according to the type of schedule given. Preferably it is about 30-60 mg/m^2/day, more preferably about 40-50 mg/m^2/day, even more preferably about 40 mg/m^2/day.

The dosage amount of carboplatin is the full dosage range used according to the type of schedule given. Preferably it is about 200-400 mg/m^2/day, more preferably about 250-300 mg/m^2/day.

In a particular embodiment, the infusion time of ET-743 is between 1 and 3 hours, preferably between 2 and 3 hours. Especially preferred is a time of about 3 hours.

The above schedules and dosages allow for an effective combination cancer therapy in humans, while avoiding toxicities. We have found that ET-743 in combination with cisplatin or carboplatin is effective in the treatment of several cancer types, including advanced or metastatic. Preferably, the combination ET-743 with a platinum compound is used according to the above schedules and dosages for the treatment of sarcoma, osteosarcoma, ovarian cancer, breast cancer, melanoma, head and neck cancer, colorectal cancer, mesothelioma, renal cancer, endometrial cancer and lung cancer.

Depending on the type of tumour and the developmental stage of the disease, the treaments of the invention are useful in preventing the risk of developing tumours, in promoting tumour regression, in stopping tumour growth and/or in preventing metastasis.

Although guidance for the dosage is given above, the correct dosage of the compound will vary according to the particular formulation, the mode of application, and the particular situs, host and tumour being treated. Other factors like age, body weight, sex, diet, time of administration, rate of excretion, condition of the host, drug combinations, reaction sensitivities and severity of the disease shall be taken into account. Administration can be carried out continuously or

periodically within the maximum tolerated dose.

EXAMPLES

Example 1

In order to evaluate the effects of the combination of ET-743 and cisplatin (DDP) *in vivo* we selected some xenografts relatively resistant to a single dose of DDP and moderately sensitive to a single dose of ET-743. For the administration of the drugs appropriate vehicles were injected, using the same schedule and route of injection as the drug therapies. ET-743 and DDP were given 1h apart in sequence or simultaneously. In xenografts transplanted s.c. tumor growth was monitored and tumor weight (TW) was determined by measuring the tumor diameters with a Vernier caliper every 2-4 days and using the formula $TW = d^2 \times D/2$ (where d and D represent the shortest and the longest diameter respectively).

The maximal single i.v. dose of DDP and ET-743 that caused no toxic death were respectively 12 mg/Kg and 0.2 mg/Kg. The same dose of each drug could be given when the two drugs were administered in combination with a tolerable toxicity, with a maximal weight loss ranging from 10 to 26 % in different experiments(n=14) with a median value of 15%. Surprisingly, the treatment with the combination caused only a slightly higher weight loss than the treatment with each drug alone. The toxicity did not appear different when the two drugs were given simultaneously or one given after the other with an interval of 1 h in either of the two sequences.

The antitumor activity of the combination was greater than that of each drug alone in all models. In TE-671 rhabdomyosarcoma and SK-N-DZ neuroblastoma all three combinations (i.e. ET-743 given 1 h before DDP or at the same time or 1 h after DDP) were compared and no significant differences in the antitumor activity were observed. Also in H & N FADU, in NSCLC LX-1, in melanoma H-187 and in the ovarian SKOV where the two sequences were compared no consistent differences related to the sequence was found.

Collectively all the data indicate that the antitumor activity of the combination was greater than that of each drug alone and the sequence does not influence the treatment efficacy and toxicity in a consistent fashion.

Example 2

The observation that the toxicity of the combination appeared very modest prompted us to test the effect of the combination of ET-743 and DDP splitting the dose of both drugs in three administrations with an interval of 4 days.

1A9 ovarian carcinoma xenografts were relatively resistant to the two drugs used as monotherapy. In contrast DDP at 4 mg/kg (Q4x3) for a total dose of 12 mg/kg given simultaneously to ET-743 at 0.1 mg/kg (Q4x3) for a total dose of 0.3 mg/kg induced a significant TWI of 73%.

Again we observed no toxic deaths or severe toxicity with the combination (mean body weight loss 16%) compared to the single drugs (14% and 12% with ET-743 and DDP respectively).

Thus the combination therapy allows high dosages and even in tumors in which the two drugs produced no significant activity given alone there was evidence of activity of the combination of each of the drugs. The combination is particularly successful in overcoming resistance in ovarian carcinoma xenograft.

Example 3

In ovarian carcinoma patients the tumor spreads into the peritoneal cavity. Therefore to mimic clinical disease we have selected a human ovarian xenograft, HOC 8, which was transplanted intraperitoneally from ascites and disseminated in the peritoneal cavity. This tumor is partially sensitive to DDP (ILS=139%) and insensitive to ET-743 (ILS=21% and 23% with 0.05% and 0.15 mg/kg, Q4x3).

When the two drugs were combined the effect was much greater than that of each drug given as single agent with a dramatic increase in survival. Both the low (ILS=258% versus vehicle) and the high (ILS=322% versus vehicle) dose of ET-743 combined with DDP increased the survival time of mice bearing HOC8, that was significantly improved compared to DDP as monotherapy (ILS=49% and 76% versus DDP with low and high dose of ET-743 respectively). Three animals were still alive after 12 months, two of them belonging to the group receiving the high ET-743 dose. They were sacrificed and a detailed macroscopic and microscopic pathological evaluation was performed. The mouse belonging to the group receiving the low ET-743 dose was apparently cured as the microscopic analysis of liver, splen, pancreas, bone marrow, diagram, ovary uterus omentum and several lymphnodes were negative. Instead both the other long term surviving mice showed a residual tumor at the level of omentum and in one of them a single

metastasis in the uterus was found, while in the other organs no metastases were detected.

This example shows the potential of the combination in ovarian cancer, even if there is metastasis.

Example 4

We designed a multicenter dose finding trial, on a day 1 & 8 every 3 weeks schedule, with escalating doses of ET given as a 3-hr infusion with steroids and antiemetic prophylaxis, followed 30 minutes later by 1 hr infusion cisplatin at a fixed dose of 40 mg/m^2, with 2 L NS hydration.

36 patients were entered (15 with ovarian cancer, 6 with uterus cancer, 14 with soft tissue sarcoma, 1 with other tumor type). Prior treatments of the patients were the following:

PRIOR TREATMENTS	
No OF PTS WITH PRIOR CHEMO FOR ADVANCED DISEASE	35
N° OF PRIOR CHEMO REGIMENS FOR ADVANCED DISEASE	median 1 *range 0-2*
N° OF PTS W/PRIOR PLATINUM	22 (61%)
• ***RESPONDERS***	*9*
• ***RESISTANT***	*13*
N° OF PTS W/PRIOR CARBOPLATIN	16
• ***RESPONDERS***	*6*
• ***RESISTANT***	*10*

ET-743 doses levels were 300, 400, 500, 600 and 700 μg/m²/day; 3-6 pts were treated per dose level according to toxicity.

ET-743 escalation was uneventful until 500 μg/m²; at 600 μg/m² patients were accrued in 2 separate risk cohort according to prior chemotherapy extent: low risk 1 regimen (LR); high risk ≥ 2 regimens (HR).

The following table illustrates the hematologial toxicity found:

HEMATOLOGICAL TOXICITY				
ET-743 D 1&8 μg/m²	Enrolled pts	Treated pts	Pts With toxicity at cy 1	
			G3	G4
300	3	3	none	none
400	3	4*#	none	none
500	8	7°	2 ANC	none
600	15	15°	8 ANC	none
700	7	7	2 ANC 1 Hb	2 ANC 1 PLT

* 7 days duration of G4 neutropenia → DLT

° 1 pt at 500 and 1 pt at 600 failed to recover from neutropenia by > d35 → DLT

1 pt enrolled at 500 received 400 at cycle 1

The following table illustrates the non-hematologial toxicity found:

NON-HEMATOLOGICAL TOXICITY BY DOSE & SCHEDULE								
ET-743 dose D 1&8 μg/m²	N° of treated pts	Asthenia		N&V			AST/ ALT	
		G1	G2	G1	G2	G3	≤G2	G3
300	3	1	1	-	-	-	1	-

400	4	1	1	-	1	-	2	1
500	7	5	1	4	1	-	6	-
600	15	6	-	12	1	-	8	4
700	7	4	1	2	3	1	5	2

The following table illustrates other non-hematologial toxicity found:

OTHER NON-HEMATOLOGICAL TOXICITY BY DOSE & SCHEDULE			
ET-743 dose D 1&8 $\mu g/m^2$	Treated Pts	G1	G2
300	3		
400	4*		1 anorexia 1 phlebitis
500	7*		
600	15	3 SNP	1 abdo cramps 1abdo pain
700	7		1 anorexia

Dose Limiting Toxicities (DLTs):

- 500: 1/7 treated pts failed to recover at day 35
- 600: 3/15 treated pts
    - 1 failure to recover hematological toxicity at day 35
    - 1 ALT grade 3 not recovered to B/L
    - 1 failure to retreat on day 8 bilirubin grade 1, ALT grade 3
- 700: 2/7 treated pts ANC grade 4 lasting > 7 days (1 pt also had concomitant Gr 4 thrombocytopenia and failure to recover hematological toxicity at day 35)

The following table illustrates the observed efficacy:

TUMOR	PRIOR TX		ET-743 dose D 1&8 $\mu g/m^2$	SITES OF DISEASE	BEST RESP	TTP mos
Ovary	carboplatin + taxol	NE	600	Pelvis, liver	PR	6+
	paclitaxel	PD				
STS gyn	adriamycin/ifosfamide	AD	700	Lung	PR	3+
Ovary	carboplatin + taxol	NC	400	Lung	PR	6
	topotecan	PD				
Ovary	carboplatin + taxol	PR	600	Liver Abdomen	Unc PR	Too early
STS-gyn	epirubicin + ifosfamide	AD	500	Abdomen	Radiological PR non measurable lesions	5+
	gemcitabine	PD				
Uterus Colon	carboplatin	NE	400	Bone, Pelvis Lung	Radiological PR non measurable lesions	1
	taxol + epirubicin + cisplatin	CR				

(PR: partial response; PD: progressive disease; CR: complete response; NC: no change; AD: adjuvant; NE: non-evaluable; TTP: time to progression)

From this study we concluded that:

- In this population the MTD is 700 $\mu g/m^2$ in previously treated patients day 1 & 8 every 4 weeks
- Recommended dose (RD) in previously treated patients is 500 $\mu g/m^2$ day 1 & 8 every 4 weeks
- The DLT is myleosuppression, particulatly neutropenia
- At doses ≥ 600 $\mu g/m^2$ day 1 & 8 every 3 weeks delayed recovery from neutropenia was observed in the majority of patients.

- Main non-hematological toxicities are dose-dependent nausea and vomits (N&V), Asthenia and Liver toxicity (always reversible and mild up to 600μg/m²/day).
- Main non-hematological toxicities were dose-dependent N&V and asthenia
- Optimum interval of re-treatment is day 28

Example 5

We designed a multicenter dose finding trial, on a day 1 every 3 weeks schedule, with carboplatin given at a fixed dose of 300 mg/m² as a 1 hour infusion followed by escalating doses of ET given as a 3 hours infusion with steroids and antiemetic prophylaxis.

11 patients were entered (6 with ovarian cancer, 1 with lung cancer, 4 with soft tissue sarcoma). Prior treatments of the patients were the following:

N of patients	11
Type of tumor	
Non Small Cell Lung Cancer (NSCLC)	1
Epithelial Ovarian Carcinoma	6
Soft tissue sarcoma	4
Previous treatment	
One line chemotherapy	6
Two or more lines	5
Previous treatment with Carboplatin	6 (all Ovarian Carcinoma)

ET-743 doses levels were 500, 650 and 800 μg/m²/day; 3-6 pts were treated per dose level according to toxicity.

The maximum tolerated dose (MTD) was defined as the highest dose level tested of the combination at which at least 2 patients experience a DLT in cycle 1. If one patient encountered drug-induced DLT during either cycle 1 or 2, up to a maximum of 6 patients could be treated at that level. If DLT was not observed in the additional patients, new patients could be treated at the next higher dose level.

The cycle 1 haematological toxicities for platelets and absolute neutrophil count (ANC) are reported in the following table:

Level	No of patients	Neutropenia				Thrombopenia			
		G0	G1	G2	G3	G0	G1	G2	G3
500	3	2	0	1	0	0	2	1	0
650	3	0	1	2	0	0	3	0	0
800	5	2	1	1	1	1	1	1	2

Two patients developed DLT during the first course with grade 3 thrombocytopenia in dose level 3. Both patients had Ovarian Carcinoma pretreated with Carboplatin.

The following table shows haematological toxicities for platelets and ANC for all the courses administered, as well as the number of cycles without haematological recovery by day 21 and 28.

ET-743 dose administration	No of courses	No of courses without haematological recovery at day + 21	No of courses without haematological recovery at day + 28
400*	4	1/4	0/3
500	13(1)	6/13	5/13

650	4[(2)]	4/4	0/3
800	9	3/6	0/5

* all after dose reduction; (1) 8 after dose reduction; (2) 1 after dose reduction

ET-743 dose administration	Neutropenia				Thrombopenia			
	G0	G1	G2	G3	G0	G1	G2	G3
400*	0	2	2	0	0	3	1	0
500	4	3	5	1	5	5	2	1
650	0	1	3	0	0	3	0	1
800	4	1	1	3	3	2	1	3

* all after dose reduction; (1) 8 after dose reduction; (2) 1 after dose reduction

And the number of patients with dose delayed (and reduced) at cycle 2 with the reason for this delay is reported in the following table for each dose level:

level	No of patients receiving Cy 2	Dose delayed (and reduced) at cycle 2	Reason
500	3/3	1 pt	ANC G2
650	3/3	3 pts	Thrombopenia G1 ANC G2 ANC G2
800	4/5* *one patient too early	2 pts	ANC G3 ANC G3

As can be inferred from the cycle 1 data from 11 patients treated:

- In this population the MTD is 800 μg/m^2 of ET-743 with carboplatin at a fixed target (300 mg/m^2)

- DLTs consist of Thrombocytopenia grade 3
- In this population at the second dose level, 100% of patients had a dose delay with dose reduction at the second cycle because of haematological toxicity
- At the third dose level, 50% of patients had a dose delay with dose reduction at the second cycle because of haematological toxicity

Given this haematological safety profile, with long lasting, though moderate neutropenia that prevent from achieving an adequate dose intensity of ET-743, and the 2 DLTs consisting of Thrombopenia grade 3 in two patients with Ovarian Carcinoma previously pretreated with carboplatin, it can be inferred the following schedule:

- In patients previously treated with carboplatin: Administration of carboplatin at a fixed target (250 mg/m^2) over 1h infusion followed by ET-743 over 3 hours iv infusion on day 1 every 3 weeks.

- In patients not previously treated with carboplatin: Administration of Carboplatin at a fixed target (300 mg/m^2) over 1h infusion followed by ET-743 over 3 hours iv infusion on day 1 every 3 weeks.

Claims

1. A method for mitigating resistance to a platinum coordination complex anti-neoplastic agent in a human cancer patient which comprises administering to the patient a platinum coordination complex anti-neoplastic agent and ET-743.

2. A method for potentiating the cytotoxic effects of a platinum coordination complex anti-neoplastic agent in a human cancer patient which comprises administering to the patient a platinum coordination complex anti-neoplastic agent and ET-743.

3. A method according to claim 1 or 2, wherein ET-743 is administered as a combination therapy with the platinum anti-neoplastic agent without a compensating drop in the dose of the platinum anti-neoplastic agent.

4. A method according to claim 1 or 2, which comprising administering to an individual the platinum anti-neoplastic agent and ET-743 in dosage amounts which are in the same range as the dosage given if each of ET-743 and the platinum compound is administered alone.

5. A method according to claim 4, wherein the amounts of the platinum anti-neoplastic agent and ET-743 are both at least at least 90% of the respective Recommended Dose.

6. A method according to any preceding claim, wherein the platinum anti-neoplastic agent is selected from cisplatin, carboplatin, tetraplatin, ormiplatin, iproplatin, oxaliplatin.

7. A method according to claim 6, wherein the platinum anti-neoplastic agent is cisplatin or carboplatin.

8. A method according to claim 7, wherein the platinum anti-neoplastic agent is cisplatin.

9. A method according to any preceding claim, wherein the platinum anti-neoplastic agent and ET-743 are administered sequentially.

10. A method according to claim 9, which comprises a step of intravenously infusing a composition comprising ET-743 into the patient at a continuous dosage over a period up to 4 hours, followed or preceded by intravenously infusing a composition comprising a platinum antineoplastic agent into the patient at a continuous dosage, and repeating weekly the steps of infusing on a cyclic basis.

11. A method according to claim 10, in which the cyclic basis comprises two phases, a phase of weekly infusing, referred to as an infusion phase, and a phase of not infusing, referred to as a rest phase,

with the the cycle comprising one or more weeks of an infusion phase, and one or more weeks of a rest phase.

12. A method according to any preceding claim, in which the patient is relapsing or refractory to previous chemotherapy.

13. A method according to any preceding claim, in which the patient has a cancer selected from sarcoma, osteosarcoma, ovarian cancer, breast cancer, NSCL carcinoma, melanoma, head and neck cancer, colorectal cancer, mesothelioma, renal cancer, endometrial cancer and lung cancer.

14. A method according to any preceding claim, in which the patient has a cancer selected from ovarian cancer, NSCL carcinoma, melanoma, head and neck cancer.

15. A pharmaceutical composition for weekly administration containing a recommended dose of a platinum coordination complex anti-neoplastic agent and a recommended dose of ET-743.

16. The use of a platinum coordination complex anti-neoplastic agent in the preparation of a medicament for a method according to any of claims 1 to 14.

17. The use of ET-743 in the preparation of a medicament for a

method according to any of claims 1 to 14.

18. A medical kit for administering an antineoplastic platinum anti-neoplastic agent in combination with ET-743, comprising a supply of ET-743 in dosage units for at least one cycle, wherein each dosage unit contains the appropriate amount of ET-743 and a pharmaceutically acceptable carrier, and printed instructions for administering ET-743 according to a dosing schedule.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB2004/002319

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/495 A61K31/282 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 02/36135 A (PHARMA MAR SA ; GESCHER ANDREAS (GB); GIAVAZZI RAFAELLA (IT); INCALCI) 10 May 2002 (2002-05-10) cited in the application claims 1,7,8 page 2, lines 11,12 examples 3,4,6,8	1-18
X	"In human tumor xenografts the resistance to ET-743 or to cisplatin can be overcome by giving the two drugs in combination" EUROPEAN JOURNAL OF CANCER, PERGAMON PRESS, OXFORD, GB, vol. 38, November 2002 (2002-11), page S34, XP004403537 ISSN: 0959-8049 abstract	1-18

-/—

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 September 2004	01/10/2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Peris Antoli, B

Form PCT/ISA/210 (second sheet) (January 2004)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB2004/002319

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE MEDLINE 'Online! US NATIONAL LIBRARY OF MEDICINE (NLM), BETHESDA, MD, US; December 2002 (2002-12), SCOTLANDI KATIA ET AL: "Effectiveness of Ecteinascidin-743 against drug-sensitive and resistant bone tumor cells." XP002296203 Database accession no. NLM12473605 abstract abstract & CLINICAL CANCER RESEARCH : AN OFFICIAL JOURNAL OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH. DEC 2002, vol. 8, no. 12, December 2002 (2002-12), pages 3893-3903, ISSN: 1078-0432 ----	1-18
P,X	D'INCALCI M ET AL: "The combination of yondelis and cisplatin is synergistic against human tumor xenografts" EUROPEAN JOURNAL OF CANCER, PERGAMON PRESS, OXFORD, GB, vol. 39, no. 13, September 2003 (2003-09), pages 1920-1926, XP004446970 ISSN: 0959-8049 abstract ----	1-18

INTERNATIONAL SEARCH REPORT

national application No.
PCT/GB2004/002319

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: –
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 1-14 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Inj tional Application No
PCT/GB2004/002319

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0236135 A	10-05-2002	AU 1249902 A	15-05-2002
		BG 107843 A	30-06-2004
		BR 0115162 A	21-10-2003
		CA 2428160 A1	10-05-2002
		CN 1486193 T	31-03-2004
		CZ 20031327 A3	12-11-2003
		EP 1365808 A2	03-12-2003
		WO 0236135 A2	10-05-2002
		HU 0400648 A2	28-06-2004
		JP 2004517056 T	10-06-2004
		NO 20032027 A	04-07-2003
		SK 5492003 A3	02-03-2004
		US 2004108086 A1	10-06-2004